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NERVE ACTION POTENTIALS OF THE DEVELOPING CHICK

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IN A PREVIOUS paper García Austt (1954) studied the electrical activity in the cerebral hemispheres of the chick embryo. It was shown that "spontaneous" activity appears on the 13th day of incubation and that the EEG pattern changes remarkably from the 16th day onwards.

It was considered interesting to study the electrical activity of an isolated nerve structure during embryonic differentiation in order to find a possible relationship between the EEG development and the physiological differentiation of a simpler nerve structure. The present paper also deals with the electrical activity of peripheral nerve up to 19th day after hatching.

MATERIAL AND METHOD

Thirty-one experiments were performed at room temperature (18°C-22°C) in Rhode Islands Red embryos and new-born chicks. The sciatic nerve was isolated in its path along the dorsal surface of the thigh and mounted on platinum electrodes. For stimulation and recording it was lifted into liquid petrolatum. After the twelfth day of incubation the potentials were recorded monotopically. Before that date the recording was usually bitopic.

The recording electrodes were at a distance of 2-4 mm from the cathode. The cathode was 2 mm apart from the anode. Potentials were registered by means of an R. C. pre-amplifier and a cathode ray oscillograph. The nerve was stimulated by rectangular pulses.

RESULTS

Characteristic of the spike

Up to 17th day of incubation the spike obtained after supramaximal stimulation was complex showing 2 or 3 deflexions (Figs. 2, 3, 7). After

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this date it became simpler. Simplification of the nerve action potential was a gradual process, thus after 10 days of incubation it showed two distinct peaks with a shock-peak time of 20 and 45 milliseconds. The latency was of the order of 12 milliseconds measured from the shock artifact to the foot of the ascending limb. The total potential duration was 50 milliseconds. On the following days the spike showed 3 deflexions. On the 12th day, for example, it had a total duration of 50 milliseconds, a latency of 7 milliseconds and the vertices were observed 10, 25 and 45 milliseconds after the artifact. After 18 days of incubation the spike

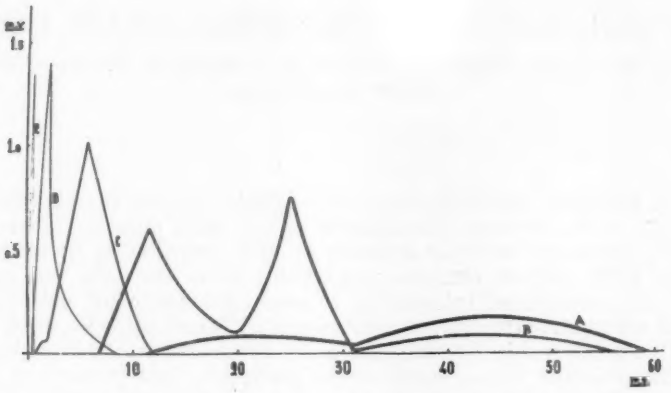

FIG. 1. — Schematic representation of the action Potentials of the Isolated Sciatic Nerve during development. *Form, duration, amplitude and latency of the spike in the course of development. Ordinate: Potentials in mv. (millivolts); Abscissa: Time in msec. (milliseconds). Square pulses of 0.001 msec were applied at o. A: 10-day chick embryo. B: chick embryo at 12th day of incubation. C: 18-day chick embryo. D: 7-day chick. E: 10-day chick.*

was single with a total duration of 110 milliseconds and a latency of 1 millisecond. After hatching, both spike duration and latency decreased progressively.

The amplitude of the action potentials also changed continuously during development, thus the maximal voltage attained at the 10th day of incubation was about 10 microvolts while after hatching it exceeded one millivolt. Figure 1 summarizes the modification in amplitude and time course produced during development both during embryonic life and after hatching.

Threshold changes were also measured in the present experiments and although the data do not allow quantitative estimates it was clear that important qualitative differences could be established during growth.

Before the 18th day of incubation, when the spike was polyphasic, the first deflexion had the lowest threshold. When the stimulus strenght was increased the second and third deflexions appeared, the threshold as a rule being lower for the second than for the third component. Figure 2 illustrates this point in an embryo of 10 days. The weakest stimulus

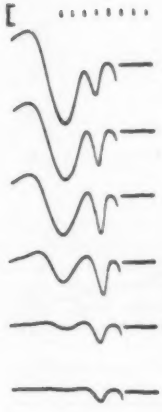

FIG. 2

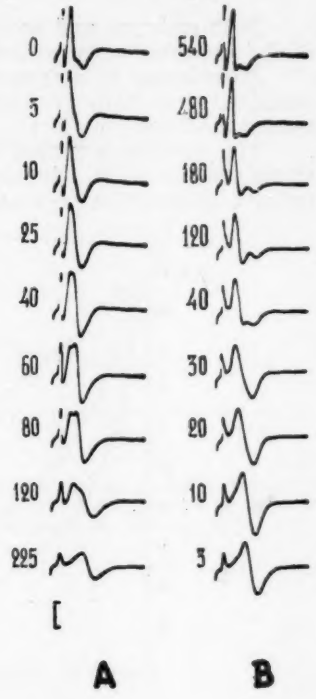

FIG. 3

FIG. 2. — Action Potential of the Isolated Sciatic Nerve of the Chick Embryo at an Incubation Age of 10 days. *From top to bottom, progressively increasing voltages. In the first record, obtained with juxtaliminar intensity, only the first deflexion of the spike appears. From the second record onwards the second deflexion appears. Last record: supramaximal stimulus. Time: 10 milliseconds: Cal: 50 microvolts.*

FIG. 3. — Fatigue and recovery of the Isolated Sciatic Nerve of 12-day chick embryo. *A: Stimulation at 5 per second. The numbers indicate time in seconds from the onset of stimulation. O: control response of a stabilized preparation stimulated at 1 per second. B: Recovery nerve stimulated at 1 per second (from bottom to top); the numbers indicate the seconds elapsed from the end of stimulation. Time 15 milliseconds; Cal: 500 microvolts.*

produced only the onset of the first deflexion while stronger stimuli brought into action the second component of the action potential. It must be noticed here that at this stage the third deflexion does not appear.

Del Castillo and Vizoso (1953) have measured the conduction velocity of the nerve action potential in chick embryos. They have reported values of less than 1m/sec. In the present experiment this observation was confirmed. After hatching the speed of conduction increased rapidly reaching 50m/sec. on the 12th day.

Fatigability of the nerve impulse

Both embryos and newly hatched chicks were employed in order to study the effect of repetitive stimulation on the nerve.

Stimulating at a frequency of 1/sec. or less the electrical response remained practically unchanged. At frequencies ranging from 5 to 20 per second modifications in the amplitude form and latency were registered.

Fig. 3 shows a typical experiment performed in an embryonic nerve stimulated at 5/sec. The amplitude of the electric potentials during repetitive stimulation decreases and the duration and latency of the dif-

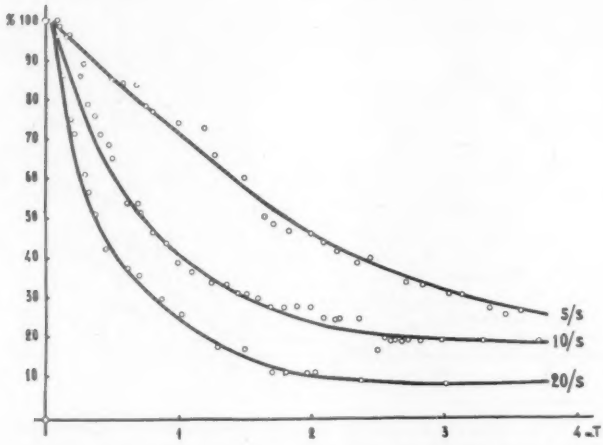

FIG. 4. — Fatigability of the Isolated Sciatic Nerve of 12-day chick embryo. *Ordinates: percentages of initial amplitude of the spike. Abscissae: time in minutes. Fatigue curves with stimuli at freguencies of 5, 10 and 20 per second.*

ferent components of the action potentials become longer. The whole picture also become simpler, i. e., the potential are less polyphasic. As it was expected, higher frequencies of stimulation induced a faster and more marked nerve fatigue (Fig. 4).

When a constant frequency was employed (e. g. 5 per sec as shown in Fig. 5) and the parameter being the days of incubation and the days after hatching, the temporal course of fatigue appears different: it is less marked as the embryo and chick grow.

Another interesting observation is the initial decline in spike amplitude recorded after 60 seconds of repetitive stimulation. The intensity of this effect depends essentially on two factors: a) the age of the embryo of the chick and b) the frequency of stimulation. If spike amplitude is plotted against days of development and the parameter is the frequency of stimulation, curves of different slopes can be obtained. Figure 6 illustrates several experiments where frequencies of 5 and 10 per second were used. The curve obtained by stimulating at 5 per sec has a slope of 0.753 while the slope of that obtained after stimulation at 10/sec is 1.072.

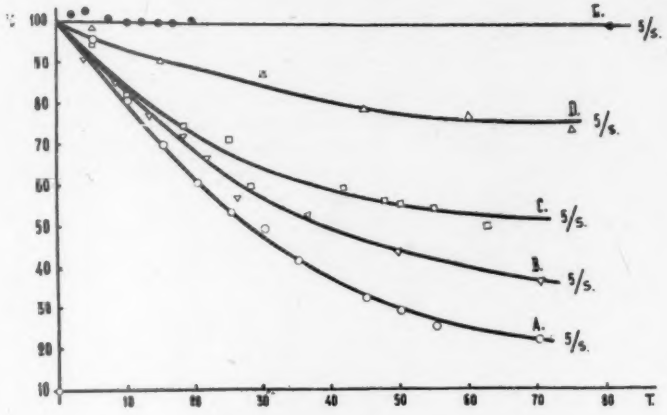

FIG. 5. — Fatigue curve of the Isolated Sciatic Nerve of the growing chick. *Ordinates: percentage of the initial amplitude of the spike. Abscissae: time expressed in seconds. The same frequency (5/sec) is used in all the curves.*
A: 11-day chick embryo. B: 17-day chick embryo.
C: 19-day chick embryo. D: 7-day chick.

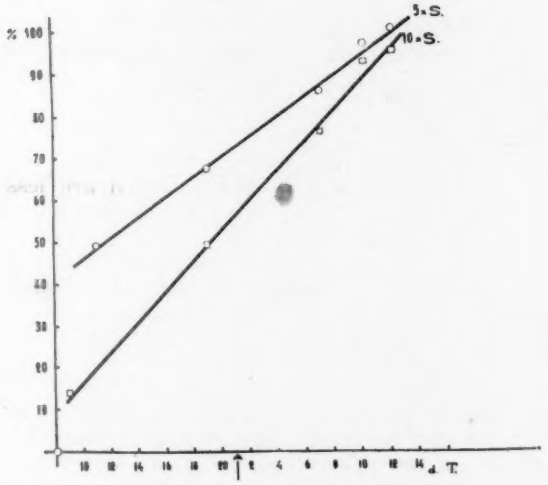

FIG. 6. — Fatigue of the Isolated Sciatic Nerve of growing chick, stimulated at frequencies of 5 and 10 per second. *Ordinates: percentage of the initial amplitude of the spike 60 seconds after starting the stimulation. Abscissae: age of the embryo and chick in days. The arrow indicates hatching.*

This indicates that during the early stages of development, fatigability is so intense that a potential may drop to 50 % of the initial values when the nerve is stimulated at 5/sec and to 15 % of the initial voltage if the frequency is increased to 10/sec. At a later stage, e g. a 12- day chick, is much more resistant to fatigue and frequency changes of this order do not show great differences.

Another set of experiments was designed in order to gather more information about the nature of the different deflexions of the action potential. A 10-days embryo was chosen and its sciatic nerve was stimulated in isolation at a frequency of 5/sec. At this stage of development the action potential may present three deflexions. The stimulus intensity was adjusted in order to stimulate all conducting nerve fibers. The typical polyphasic spike was obtained. As it may be seen in Fig. 7 A, the three components fatigued independently. Although all deflexions declines in height the third component was disappeared earlier and no traces of it could be detected 30 seconds after the onset of stimulation. The first peak was considerably reduced 30 seconds afterwards. The second component disappeared 120 seconds after stimulation begun. Stimulation was continued up to 360 seconds. After that period of time, single pulses were delivered at 1 per second. (Fig. 7, B from bottom to top) and the course of recovery was followed up to 840 seconds. It may be seen that recovery is complete. It is important to emphasize here that latency and shock-peak time increase during fatigue and that are reduced to normal values during recovery.

Figure 7, C illustrates the fatigue time course of the first component only. The stimulating voltage was adjusted in such a manner as to stimulate those fibers which produced the first deflexion. Stimulation at 5/sec was continued up to 360 seconds. At the onset a single, diphasic spike appeared. As stimulation was continued a secondary deflexion started to dissociate from the first because of an increased shockpeak time. The secondary deflexion disappeared between 180-240 seconds after the onset of stimulation, 360 seconds later only a small monophasic potential remained. Immediately afterwards the stimulus intensity was increased to values similar to these used in the records of Fig. 7, A and shocks were delivered at a frequency of 1/second. It may be seen that the first deflexion recovers rapidly and that the second and third components are very prominent (Fig. 7, D).

Recovery from fatigue was always gradual and complete (Fig. 3 and 7) in the experimental conditions used here. Usually the time was shorter the more developed was the embryo or the chick.

Discussion

The possibility of recording the electrotonic spread of the local potential instead of a propagated action potential with the electrode placed 2 to 4 mm from the cathode is excluded because when polarity of the stimulating electrodes was reversed the response showed no alteration in its amplitude or shape, only the latency increased. In other words, it was conducted with no decrement i. e., it was an all-or-none response.

During the growth of the cat, Hursh (1939) has described the characteristics of the spike. At 4 days a spike of 2 components was present

and after 8 days a third component appears. The results reported here are similar in the sense that during the 10th day of incubation only two components of the spike were present. Later on, three deflexions appear using the same speed. Near the end of the incubation period a small and a large component form the response of the nerve. After hatching a simple spike is recorded. These results can be interpreted as follows: the different groups of fibers in a sciatic nerve might not start conducting at the same time and during growth and differentiation they tend to superimpose their responses due to changes in their conduction velocity.

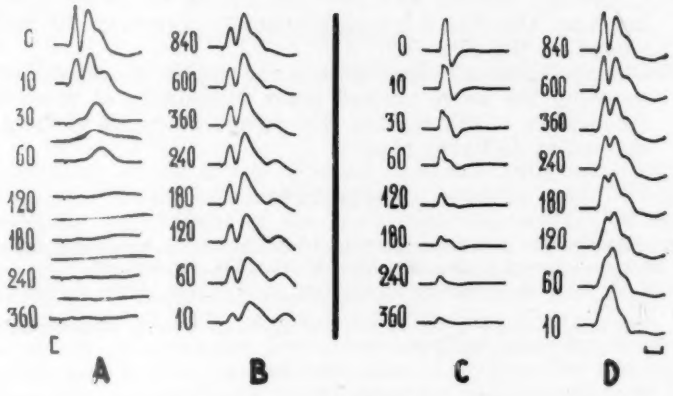

FIG. 7. — Independent fatigue of different deflexions. *17-days chick embryo. Frequency of stimuli 5 per second. A: maximal nerve stimulation. Numbers indicate time in seconds from the onset of stimulation. B: recovery from fatigue produced in A: and tested with maximal stimulation at a frequency of 1/second. Record from bottom gives in seconds the time elapsed from the end of stimulation at 5/sec. C: same as in A but the intensity of the stimulation is maximal for first deflexion only. D: same as in B applying stimuli intensity as in A and B in order to observe the unfatigued second and third deflexions of the spike. Time: 20 milliseconds. Cal: 200 microvolts.*

In other words, conduction velocity could be used as an index of maduration because younger fibers conduct at a slower rate.

Besides, different groups of fibers present diverse electrical excitability and diverse fatigability. Thus the different components of the spike can be obtained independently.

Del Castillo and Vizoso (1935) reported that the electrical activity of the sciatic nerve of chick embryos can be recorded from the 9th day of incubation and the medullated fibers were first observed during 15th day of incubation. Our results confirm these observations, in the sense that conduction starts before the 15th day of incubation.

It seems important to emphasize the great fatigability of the nerve during embryonic development and during the first days after hatching. This fact suggests, in some way, that speed of metabolic processes is slower, in other words, that a metabolic safety factor is lower the younger is the embryo or the chick.

Summary

1 — The nerve action potentials in the embryo (from an incubation age of 10 days) and in the post-natal chick up to 19 days were studied.

2 — The action potentials were polyphasic up to an incubation age of 17 days.

3 — The duration and latency of the spike decreased and the amplitude increased steadily with age, both in the embryo and in the chick.

4 — Conduction velocity was less than 1m/sec in the embryos. After hatching this value increased rapidly, reaching 50 meter per second on the 12th day.

5 — During embryonic development and during the first days after hatching the nerve showed great fatigability at relatively low frequencies of stimulation. The temporal course of fatigue was studied at different ages.

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POSITIVE INOTROPIC RESPONSE OF AMPHIBIAN HEARTS TO ADENOSINE NUCLEOTIDES

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IN THE STUDY of the action of blood incubates with snake venom on the heart of Brazilian amphibia, it was observed that they induced an increase in the strenght of the heart contractions (1). The same reaction was also observed when the hearts were treated with tissue extracts. Some physical-chemical properties of the active substance in the incubates and tissue extracts pointed to adenosine nucleotides, released from tissues, as the substances probably responsible for this heart effect.

The action of adenosine derivatives on European frog's heart has been investigated by many workers, but the results reported are somewhat misleading. Parnas and Ostern (9, 10) observed that adenylic acid (AMP) and adenosine triphosphate (ATP) provoked in perfused frog's heart at first an increase in the height of the amplitudes, which was then followed by heart block. The same authors (10) found that the heart positive response was better elicited in exhausted organs. These workers found that other components of the adenosine group exert only blocking action upon the heart (8, 10). Lindner and Rigler (7) reported a positive inotropic effect observed with ATP in hearts rendered hypodynamic by oxygen want. Gillespie (4) has described only blocking actions with this group of substances, and Lichtneckert and Straub (6) were able to recover with ATP the frog's heart depressed by quinine.

Considering the facts reported before, it became necessary to study the actions of adenosine derivatives on hearts of Brazilian amphibia, thus permitting the application of these results for the identification of the tissue substances, the activity of which on these animals'hearts had been observed.

In this paper, data on the actions of adenosine nucleotides and other adenosine derivatives on perfused hearts of Brazilian toads and frogs are presented.

EXPERIMENTAL

The perfusion. — Hearts of toads *(Bufo marinus)* and frogs *(Leptodactylus pentadactylus and L. ocellatus)* were employed, and from these,

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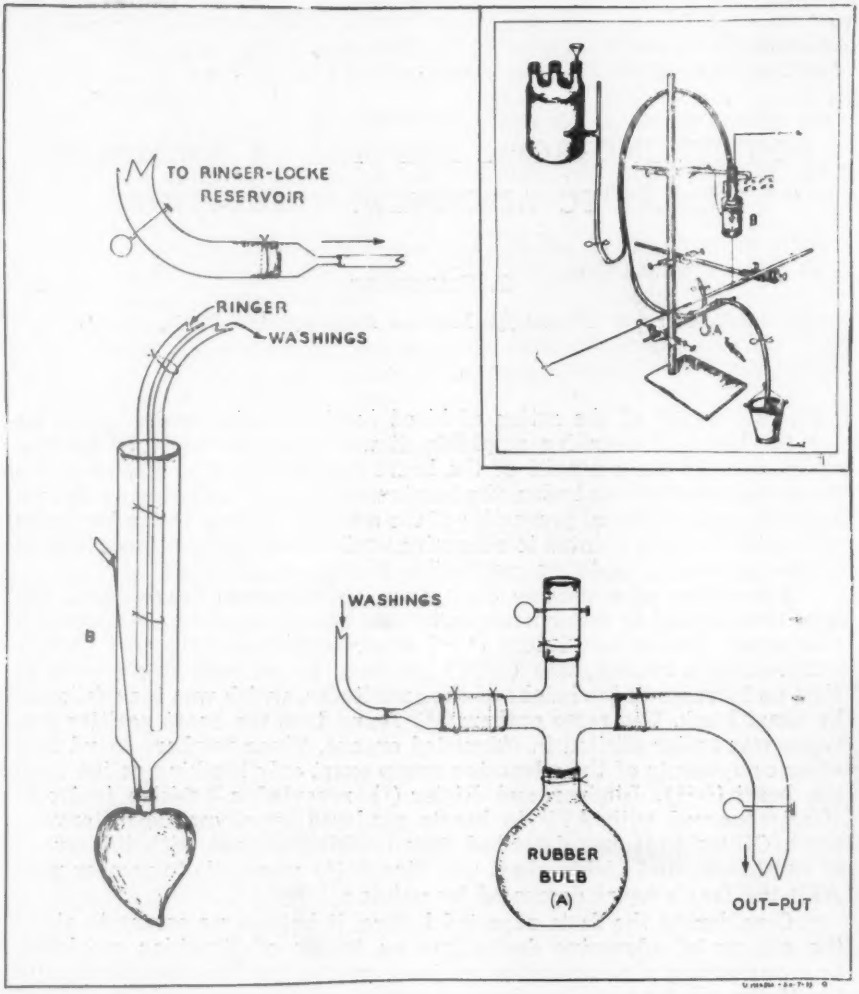

FIG. 1. — *Schematic drawing of perfusion apparatus for amphibia hearts. Upper right, assembled apparatus. (A) rubber bulb and glass connection to remove air bubbles and fill the syphoning tubes. B arrangement of the plastic tubes in the cannula.*

the toad heart was the most frequently used in the present investigation. After pithing the animal, the heart was rapidly cannulated through the aorta with a Straub cannula and then removed. Ringer-Locke solution for frogs was used, the hydrostatic pressure in the cannula being maintained constant in all experiments. Oxygen was continuously bubbled through the solution in the cannula and a glass sleeve was used to keep the heart

moist. After preparing the heart, an interval of one hour was always allowed to pass before beginning the experiments, when the heart attains a good sensitivity.

Perfusion apparatus. — The apparatus of Fig. 1 has been designed to simplify, in long heart perfusion work, washing and renewing of the

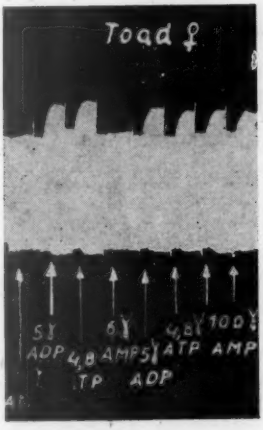

FIG. 2. — *Responses of perfused toad heart to adenosine nucleotides. The intensity of response to AMP is smaller than to ADP and ATP.*

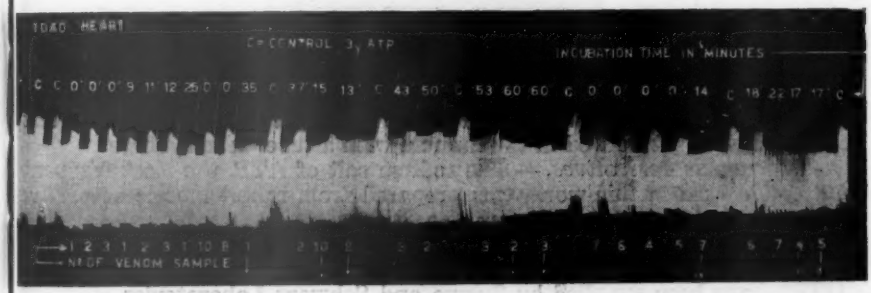

FIG. 3. — *Perfused toad heart. Continuous assays with ATP in which long intervals were allowed between tests.* 3_8 *ATP was used as control (C). The heart maintains the same reactivity to the control in all assays. The record is one determination of enzymatic degradation of ATP in heart inactive molecules by several samples of snake venoms* (Crotalus t. terrificus). *The arrows mark the assay in which the numbered incubate turned inactive to the heart by the action of venom enzymes.*

solution in the cannula. Two transparent plastic tubes, 3 millimeters in diameter, such as those used in electronics for insulation, are joined together with thin thread (Fig. 1 B). One of the tubes is used to fill the cannula by hydrostatic pressure and the other for washing it, the

latter working as a syphon. The washing tube is two centimeters longer than the other one and, when pressed against the bottom of the cannula, permits to empty, by aspiration, the liquid from the heart especially when it is not beating. After each test, the two tubes are introduced in the cannula and by means of them the heart can be rapidly washed and the cannula refilled. A four-way glass connection with a rubber bulb is intercalated in the syphoning tubes for filling the system at the start of the experiments and to remove air bubbles from it (Fig. 1 A). Oxygen is

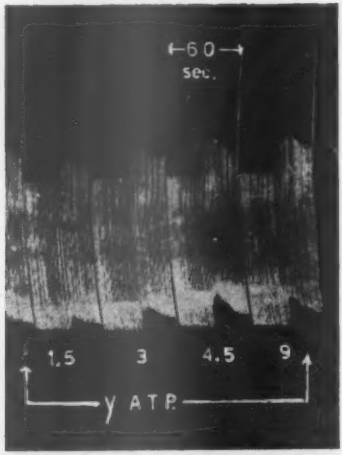

FIG. 4. — *Responses of perfused toad heart to increasing doses of ATP.*

bubbled through the cannula solutions by a glass capillary. The test solutions were introduced in the cannula with a tuberculin syringe.

Adenosine derivatives. — The sodium salt of ATP, the most frequent nucleotide used in this work, was prepared from rabbit muscle according to LePage's description ([5]), and showed by chromatography a slight contamination with AMP.Chromatographically pure adenosine sulphate, AMP, sodium adenosine diphosphate (ADP) and sodium inosine triphosphate (ITP) have been supplied by Sygma and Schwarz Laboratories.

RESULTS

Heart response to adenosine nucleotides. — The heart responds promptly to ATP, ADP and AMP with an increase in amplitude as shown in Fig. 2. The heart attains its best sensitivity after it has been maintained in perfusion for one hour approximately. In many instances the heart does not react well before this interval, even with large doses. During this stage, the sensitivity of the heart increases gradually and then remains constant for hours if sufficiently large interval is given between assays (Fig. 3). The heart is generally more sensitive in the first tests,

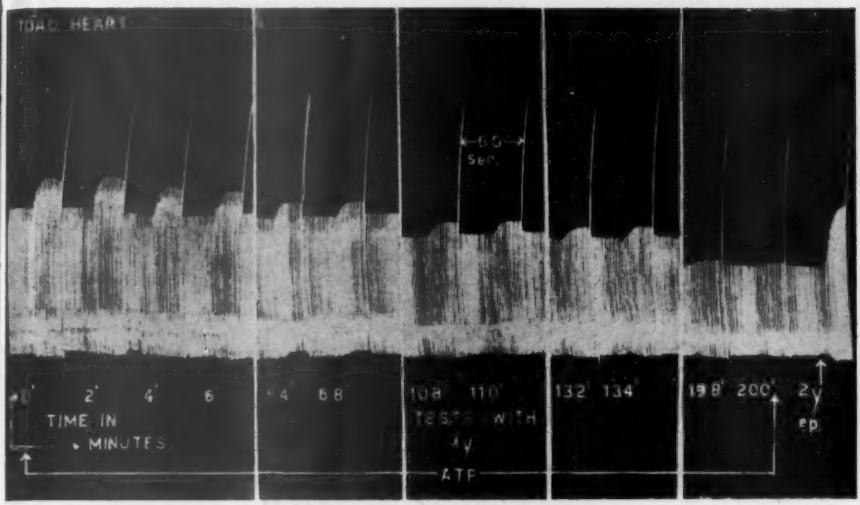

FIG. 5 — *Desensitization of toad heart to ATP in continuous tests with the same substance, 2 minutes interval were given after each test. The last test was made with 2 $_{\gamma}$ epinephrine (ep.) when the heart was already desensitized to ATP.*

where higher amplitudes are obtained with the same doses and where also the threshold of sensitiveness is lower. Although the sensitivity of the reaction varies from animal to animal, most of them give good responses with 0.5 γ ATP in summer. Tachycardia is observed in assays where alterations in rhythm occur. The heart reaction is always of the same type, varying however, in degree proportionally to the dose (Fig. 4). Continuous tests with short intervals gradually desensitize the heart until a response can no more be elicited even with large doses (Fig. 5). In this stage, the hearts still react to epinephrine(Fig. 5) but their reactivity to ATP cannot be restored by treatment with epinephrine, digitoxin or ouabain. Single tests with very large doses do not desensitize the heart to subsequent assays (Fig. 7). The responses to ATP and ADP are of the same degree (Fig. 2), whereas AMP gives responses that are approximately 20 times smaller than those observed with the other two nucleotides (Figs. 2, 6).

Seasonal refractoriness. — From April to August, the amphibian hearts employed gradually lose their susceptibility, until they become entirely refractory to the nucleotides. This phenomenon has been observed during three consecutive years. The period of refractoriness may be somewhat longer or shorter depending on the lenght of the winter. This refractory state is not directly related to environment temperatures but to a kind of semi-hibernation shown by these animals. When these amphibians enter in a motionless state, the heart reaction can no more be elicited, this fact is observed some time before the temperature fall in

winter occurs and the heart reaction starts again when the temperature is still low. In these months, the heart response to adenosine nucleotides cannot be obtained even on exceptionally warm days or after keeping the animals for 30 days at 37° C or by previous treatment with epinephrine, digitoxin or ouabain. Epinephrine remains effective in this period of refractoriness to adenosine nucleotides.

The action of other adenosine derivatives. — The increase in amplitude could only be induced with adenosine nucleotides. All the components

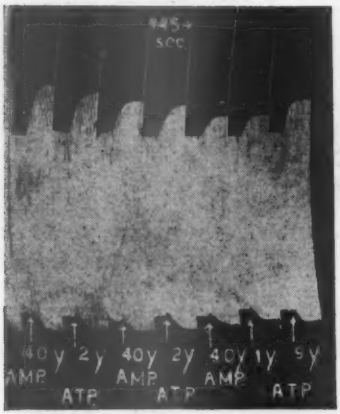

FIG. 6. — *Intensities responses of toad heart to ATP and AMP. ATP responses are approximately 20 times more intense than those obtained with the same dose of AMP.*

of the adenosine group studied failed to elicit heart block in the amphibian species of the present investigation, even using very large dosis (Fig. 7). Adenosine, which is described by some workers as the most active blocking component of this group, also does not induce heart block in these animals (Fig. 8); ITP does not increase the heart amplitude and has only a slight depressing action on this organ.

Epinephrine and the nucleotides action. — The positive inotropic action observed with adenosine nucleotides differs in many aspects from that of epinephrine. The nucleotides act more promptly on the heart than epinephrine; and after washing the heart recovers more rapidly from nucleotides than from epinephrine. Furthermore, the nucleotides rarely provoke tachycardia which is a common reaction to epinephrine. As stated before, the heart responds to epinephrine in winter months or after continuous tests with adenosine nucleotides when it is refractory to these substances (Fig. 5). In general, the heart responses to epinephrine are stronger than those maximally obtainable with the nucleotides (Fig. 9). Ergotamine could not be used to accentuate these differences because it does not generally block the action of epinephrine on the heart of these amphibians.

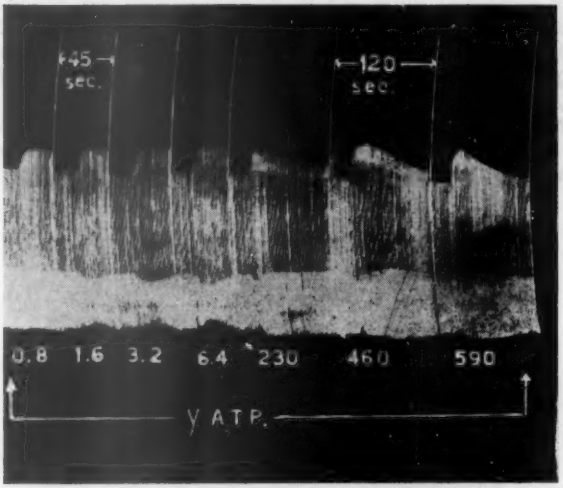

FIG. 7. — *Response of toad heart to large doses of ATP. The very large doses do not desensitize the heart to subsequent tests and also heart block is not induced with these doses.*

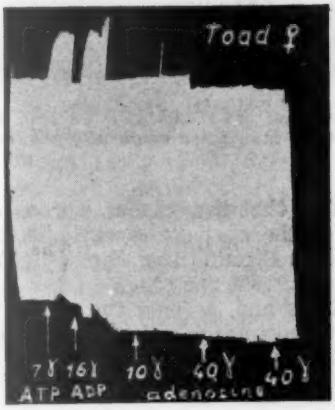

FIG. 8. — *Action of ADP, ATP and adenosine on perfused toad heart. Adenosine does not provoke heart block or positive inotropic responses.*

Pharmacological action and molecular structure. — The phosphate ribose bond is essential for the positive inotropic action, as only the nucleotides have this activity. A reinforcing action can be ascribed to the AMP-ADP bond, as it is evident from the fact that ADP acts more intensely than AMP. The last ATP phosphate bond is without pharmacological significance since ADP and ATP exert about the same action

on the heart (Fig. 2). The purine amino link of the nucleotides is also essential for the positive inotropic action, because this is not elicited by ITP. Phosphates (mono and di-sodium and sodium pyrophosphate), even in higher quantities than those probably liberated from active doses of nucleotides, are ineffective on the heart.

The autonomic nervous system and the nucleotides action. — The increased heart amplitudes induced by the nucleotides are not influenced by previous treatment of the heart with hexamethonium or atropine. On

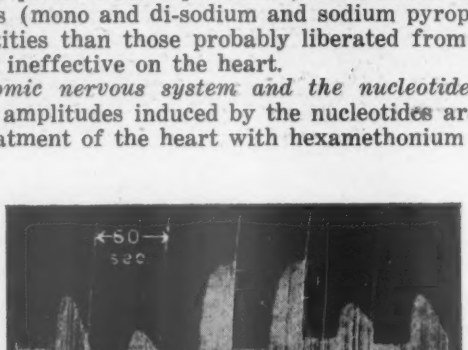

FIG. 9. — *Action of epinephrine (ep.) and ATP on toad heart. Epinephrine usually induces higher responses than those maximally obtained with ATP.*

the other hand, the fact that the cardiac actions of epinephrine are not identical with those of the nucleotides excludes the possibility that the latter exert their actions through the autonomic nervous system. It may be assumed, by exclusion, that the cardiac fiber is directly stimulated by the adenosine nucleotides and, as only the ventricular contractions were recorded, the positive inotropic action is a response of the ventricular muscle to the adenosine nucleotides.

DISCUSSION

The action of adenosine derivatives upon the heart of Brazilian amphibia differs from that reported for hearts of European frogs. In the latter, decrease in heart conduction is with rare exceptions the predominant effect observed. Positive inotropic action was also reported by some workers ([9, 10, 8]) for adenosine nucleotides beyond the blocking effects of these substances. These workers found that the positive inotropic effect is obtained in exhausted hearts or when this organ is rendered hypodynamic by oxygen deficiency, interpretations that do not fit in

our observations. In the Brazilian animals, only the positive inotropic action of adenosine nucleotides can be induced, the blocking action, which is the outstanding characteristic in European frogs ([9, 8, 10, 6]), could not be observed in the Brazilian amphibia investigated. The strength of heart contractions in our perfusions and the normal reactivity to epinephrine show that the heart function is not depressed, and thus it seems improbable that the positive inotropic effect should depend on heart exhaustion or oxygen deficiency. Also the fact that the heart becomes refractory to the nucleotides after continuous assays with small intervals does not confirm this assumption. In this particular case, it should be expected that the organ would be more depressed and then the nucleotides more active. Furthermore, the heart refractory state during winter cannot be explained by exhaustion or oxygen deficiency, as the perfusion conditions were identical to those in which the organ presents good reactivity to the nucleotides.

The observations show, as mentioned before, that the heart must be maintained approximately one hour in perfusion before it starts to respond or attains a good sensitivity to the nucleotides. Thus it must be assumed that the positive inotropic heart response to the nucleotides is a phenomenon dependent upon the perfusion. It seems probable that some heart metabolite is liberated under these experimental conditions which would give a particular heart sensitivity to the nucleotides. Continuous tests, probably modifies the cycle releasing the metabolite, thus rendering the heart refractory to the nucleotides. Some endocrine mechanism must also be involved in the heart response to the nucleotides which would explain the winter refractoriness.

It was previously reported ([3, 2]) that Brazilian amphibia reacts quantitatively or qualitatively in a different way to curare, atropine and digitalis, when compared with the same animals from other regions. The cardiac activities of adenosine derivatives presented in this paper show, therefore, one more example of the diversity in pharmacological behaviour to drugs of these animals.

SUMMARY

1 — Adenosine nucleotides induce a positive inotropic response on the hearts of Brazilian frogs and toads.

2 — Adenosine and its derivatives do not provoke heart blocking actions on these animals.

3 — The positive inotropic action of adenosine nucleotides is not observed in winter months; after continuous tests with these nucleotides, with small intervals, the heart becomes refractory to them.

4 — The ribose-phosphate and amino-purine bonds of the nucleotides are essential for the heart positive inotropic effect; the second phosphate bond has a reinforcing effect and the last phosphate bond of ATP is without pharmacological significance.

5 — The cardiac actions of nucleotides differ from those produced by epinephrine. The positive inotropic action is not exerted through the autonomic nervous system but by direct action upon the ventricular muscle.

6 — Exhaustion or oxygen deficiency does not explain the positive action of nucleotides upon the hearts of Brazilian amphibia. The mechanism of this action has been discussed and a hypothesis is put forward suggesting the liberation of a metabolite, in perfused hearts, which would sensitize the heart to these substances.

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EFFECT OF VASOPRESSIN AND OXYTOCIN ON THE EXCRETION OF WATER AND ELECTROLYTES (Na, Cl AND K) IN THE ALBINO RAT

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PREVIOUS investigations have shown that the diuretic and natriuretic effects produced by ox neurohypophyseal extracts are not modified under the action of trypsin, either alone or associated to carboxypeptidase, enzymes which abolish the vasoconstrictor potency of the extracts. These results led to the hypothesis that the diuretic as well as the natriuretic effects were due to oxytocin which, unlike vasopressin, resists the effect of those enzymes. The hypothesis would be in concordance with the results obtained by Fraser (1942) who observed that a purified oxytocin preparation stimulates the water and NaCl excretion in the rat; however, all the other references in the literature reveal discordant, results as some of the authors have attributed to vasopressin a favorable action on the excretion of Cl (Starling, 1925) and Na (Corey, 1941, Shannon, 1942; Anslow et al., 1948; Sartorius et al., 1949; Karvonen, 1952); others have been doubtful about these effects (O'Connor 1950; Sinclair, Smith et al., 1950; Chalmers et al., 1951; Murphy et al., 1951). The different experimental conditions as well as the use of posterior pituitary commercial extracts containing different proportions of both principles, might explain these discrepancies.

The availability of highly purified vasopressin and oxytocin preparations and the possibility of essaying on them the action of crystallized proteolytic enzymes allowed us to confirm the separate action of both these hormones on diuresis and electrolyte excretion (Na, Cl, K).

METHODS

An oxytocin preparation containing 240 oxytocic units and 3 pressor units per ml and vasopressin preparation containing 60 pressor units and

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3 oxytocic units per mg * were used. Albino rats normally hydrated and hyperhydrated were used.

I — *Experiments in normally hydrated animals*

Adult rats of both sexes, weighing 180 to 250 g were placed in metabolism cages and injected subcutaneously with 25 to 100 mU of vasopressin and oxytocin in 0.2 ml of a 0.9 % NaCl solution. Simultaneously, other animals were injected with oxytocin and vasopressin solutions to which trypsin (0.15 mg per 100 mU in 0.2 ml), chymotrypsin (0.15 mg per 100 mU), and trypsin plus carboxypeptidase in the proportion of 0.3 mg per 100 mU were added. The pH of the solutions with or without enzymes was adjusted to 6.8 -7 and incubated at 37°C, during 6-12 hours before being employed. Fifty animals were injected with each one of these solutions. Groups of animals receiving a saline solution which contained the respective enzymes were used as controls.

During the experiments all the animals were kept under fasting but had free access to water. The excretion of water, Na, Cl and K was determined and the values found were referred to 100 g of body weight. Controls were checked every 2 hours since 4 hours before the injection and during the first 12 hours following injections. A similar experiment was carried out in 30 rats receiving NaCl in the drinking water (0.25 and 0.5 %).

The activity of each of the solutions employed was essayed in the isolated uterus as well as in the blood pressure of rats under dial anesthesia.

II — *Experiments in hyperhydrated animals*

Sixty three rats were used, under fasting, receiving water by stomach tube in a quantity equivalent to 5 % of the body weight. During the following 2 hours, the volume of urine excreted was measured each 15 minutes. The Na concentration was determined in each sample of urine collected. A 0.1 ml saline solution containing 50 mU of oxytocin was injected subcutaneously to 33 animals 15 minutes before hydration. The rest of the animals, used as controls, were given the same quantity of saline solution but without hormone.

Results

A significant diminution in the quantity of urine and Na excreted was observed in normally hydrated rats injected with a vasopressin preparation. This fact was very clear in the first 120 minutes (fig. 1). The diminution observed in the Cl and K excretion was not statistically significant (fig. 2). During the incubation of the vasopressin preparation with trypsin or chymotrypsin, the vasoconstrictor effect of the hormone was destroyed but not its antidiuretic and antinatriuretic action. However, by association of trypsin and carboxypeptidase, this preparation lost its antidiuretic and antinatriuretic effect (fig. 1). The administration of oxytocin produced an opposite effect to vasopressin as a marked increase in the excretion of water, sodium (fig. 1) and chloride (fig. 2) was observed. This effect was specially significant in the rats receiving Na Cl in the drinking water. Diuresis and natriuresis were highly increased during

* The authors express their acknowledgement to Parke Davis & Co. for the generous supply of purified oxytocin and vasopressin preparations.

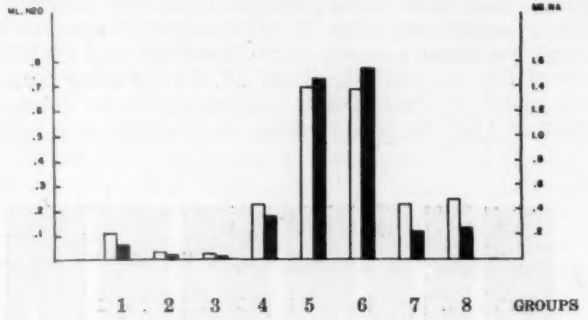

FIG. 1. — *Excretion of water and Na in albino rats with free access to water, after subcutaneous administration of vasopressin and oxytocin.*
White bars: *ml of water;* black bars: *mg of Na excreted per 100 g of body weight 120 minutes after extracts administration.*
1) vasopressin incubated without enzymes (75 rats)
2) „ „ with trypsin (22 rats)
3) „ „ with chymotrypsin (22 rats)
4) „ „ with trypsin associated to carboxypeptidase (28 rats)
5) oxytocin incubated without enzymes (122 rats)
6) „ „ with trypsin (59 rats)
7) „ „ with chymotrypsin (22 rats)
8) Trypsin and carboxypeptidase solutions in the same proportions (145 rats)

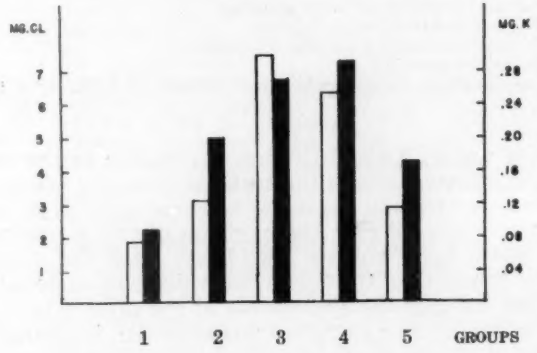

FIG. 2. — *Excretion of Cl and K in albino rats after subcutaneous administration of purified vasopressin and oxytocin preparations. During the experiments the rats were allowed free access to a 0.25 % NaCl solution.*
White bars: *mg of Cl;* black bars: *mg of K excreted per 100 g of rat during 120 minutes after administration of extracts at doses of 25 and 50 mU.*
1) vasopressin incubated without enzymes (28 rats)
2) „ „ with trypsin associated to carboxypeptidase (26 rats)
3) oxytocin incubated without enzymes (44 rats)
4) „ „ with trypsin associated to carboxypeptidase (36 rats)
5) 0.9 % NaCl with enzymes in the same proportion (62 rats)

the first 2 hours but fell in the third and fourth hour following the hormone administration. The increase observed in the excretion of K was not statistically significant (fig. 2). Incubation of oxytocin with chymotrypsin caused the disappearance of these effects and abolished the oxytocic potency of the solution (fig. 2 and 3). On the other hand, incubation with trypsin alone or associated to carboxypeptidase did not affect the oxytocic potency (fig. 3) maintaining, in addition, the increase in the

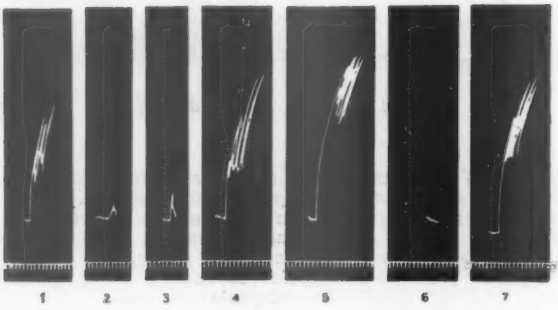

FIG. 3. — *Contractions recorded in the isolated uterus of the rat, in oxygenated Tyrode suspension.*
Added to the bath:
1) 50 mU vasopressin without enzyme.
2) 50 mU vasopressin with trypsin
3) 100 mU vasopressin with chymotrypsin
4) 0.5 mU oxytocin without enzyme
5) 2 mU oxytocin with trypsin
6) 100 mU oxytocin with chymotrypsin
7) 1 mU oxytocin
All these mixtures were incubated at pH 6.8 - 7.4 during 6 to 8 hours.

excretion of water, Na and Cl ions. No change in the excretion of water and electrolytes was seen in the controls receiving saline solution, trypsin, chymotrypsin or trypsin associated to carboxypeptidase at the same doses.

In hyperhydrated rats, the oxytocin injection produced a marked increase in the urinary volume. Two hours later, the quantity of urine excreted was larger than the volume of water administered by stomach tube while in the controls the elimination at the same time was 75.7 % ± 2.6. Changes in the sodium excretion were parallel to changes in the elimination of water. In the group of animals injected with oxytocin, the quantity of sodium excreted during the first 120 minutes, was almost twice as that found in the controls (fig. 4).

DISCUSSION

The effects produced by purified oxytocin (25 to 100 mU) in the normally hydrated albino rat are in agreement with those observed by Fraser (1942) and confirm the results we described using ox posterior

pituitary extracts (Rosas et al., 1955). The action exerted by oxytocin in rats under water overload, both on the urine volume and on the Na excretion, is similar to that produced by the injection of renin in rats under the same conditions (Croxatto et al., 1955).

This property of oxytocin may explain the polyuria seen under certain conditions with neurohypophyseal total extracts. If this effect of oxytocin on the excretion of water and Na appears in physiological conditions, it would mean that with respect to renal excretion the neurohypophysis function is opposed to that of the corticoadrenal hormones.

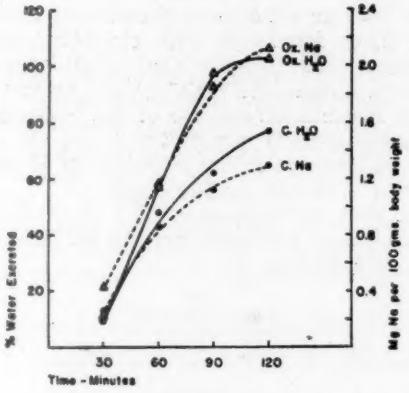

FIG. 4. — *Excretion of water and Na in hyperhydrated albino rats, during 120 minutes after administration of 50 mU of oxytocin.*
Excretion of water; excretion of Na (broken lines)
Groups indicated "Ox" received 50 mU of oxytocin (33 rats)
Groups indicated "C" are controls injected 0.1 ml NaCl 0.9 % (30 rats)

Purified vasopressin, in opposition to commercial extracts, always produces a diminution in the excretion of water and Na. Both the inhibitory action of vasopressin and the stimulating effect of oxytocin are relatively short lasting as they were only observed during the first 2 hours following injections. Probably, the neurohypophyseal secretion of the injected animal plays a part damping the effects induced by the preparations injected.

The fact that the antidiuretic potency of vasopressin is not abolished by previous treatment with trypsin or chymotrypsin, shows that the vasoconstrictor effect is independent of the antidiuretic one. Only the association of trypsin and carboxypeptidase destroys both properties. The possibility exists that trypsin and chymotrypsin do not completely destroy the molecule, releasing only some aminoacids necessary for the vasoconstrictor effect and leaving a polypeptide which keeps still the antidiuretic effect. It is also possible that the organism may rebuild the hormone using the resulting peptidic fragments.

SUMMARY

A purified oxytocin preparation, containing 240 oxytocic U and 3 pressor U per ml, injected to albino rats at doses of 25 to 100 mU produced an increase in the excretion of water, Na and Cl during 120 minutes after its administration. This property was maintained even after incubation with trypsin alone or associated to carboxypeptidase. After treatment with chymotrypsin this effect on the excretion of water, Na and Cl disappeared parallelly to the uterus contracting action.

In hyperhydrated rats, 50 mU of oxytocin produced an increase in the excretion of water and sodium almost twice as large as that observed in the controls.

A vasopressin preparation, containing 60 pressor U and 3 oxytocic U per mg injected in similar conditions showed an antidiuretic and antinatriuretic action. When incubated with chymotrypsin or trypsin these properties were maintained in spite that these enzymes destroyed the vasoconstrictor action. Treatment with trypsin associated to carboxypeptidase abolished the hypertensive effect of the preparation as well as its antidiuretic and antinatriuretic properties.

No significant differences in the excretion of K were observed in the animals injected with different preparations.

The possibility that oxytocin may play an antagonic role to vasopressin and corticoadrenal hormones on the excretion of water and sodium by the kidney is discussed.

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METHOD TO DIAGNOSE THE HEMOPHILIC CARRIER CONDITION

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THE RESULTS obtained in the investigation of the vasoconstrictor action of vasopressin on the arterioles of toads ([2]), prove that this action can be present or absent depending on what has been previously injected to the toad. Glomerule circulation stops in a few seconds if normal human plasma circulates, and do not stop, or have a long latent period if hemophilic plasma is injected.

These results suggest the possibility of the normalization of the vasopressin test of the hemophiliac by mixing normal and hemophilic plasma and also the investigation of the hemophilic carrier plasma with the same technique.

If carrier plasma does not normalize the hemophilic plasma, this test represents a method to diagnose the carrier people.

METHODS

In toads injected as previously described, the functioning of kidney glomerules was observed. To check the capacity of normal and of hemophilic carrier plasmas to normalize the vasopressin test of hemophiliacs, one part of normal or carrier heparinized plasmas, and different amounts of hemophilic plasma were mixed immediately before being injected.

The latent and the contraction periods were then observed. Latent period means the time elapsed between the vasopressin (Pitressin P. D.) instillation on the kidney, and the contraction of the glomerules as has been defined previously. Two drops of Pitressin with ten vasotonic unit per ml were instilled.

In other experiments, normal, carrier and hemophilic plasmas were diluted to 25 % with saline and separately injected in toads with the same technique.

RESULTS

Capacity of normal and carrier plasma to normalize the hemophilic vasopressin test.

1.5 ml of a mixture of 1 part of normal or carrier plasma, and 9, 19, 39, 49, or 59 parts of hemophilic plasma were injected into the

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abdominal bag of toads. Thirty minutes later, the urethane anesthesia was made, and after another 30 minutes, the vasopressin test was performed.

Mixtures of normal and hemophilic plasmas were tried from 1/40 to 1/60 dilutions. Only one case had a latent period of 150 seconds (1/40 dilution). In this case, the same test was performed with a higher concentration of normal plasma (1/20).

TABLE I

Ability of normal and carrier human plasma to normalize the vasopressin test of hemophiliac "compensation test"

Latent period (Sec.)

Nº	Normal	Carrier	Hemophiliac	Hemophiliac Plus						
				Normal				Carrier		
				1/20	1/40	1/50	1/60	1/10	1/20	1/40
I	15	15	1080	—	10	—	—	—	—	900
II	12	35	1020	—	14	—	—	—	—	780
III	14	150	N. C.	—	15	—	—	—	600	N. C.
IV	20	—	N. C.	50	150	—	—	—	360	N. C.
V	15	20	N. C.	—	15	—	—	—	—	1080
VI	180	20	N. C.	—	10	180	—	—	900	840
VII	180	15	N. C.	—	15	25	—	—	N. C.	N. C.
VIII	20	15	N. C.	—	22	—	N. C.	N. C.	—	N. C.
IX	180	20	N. C.	—	—	15	N. C.	N. C.	—	—
X	20	15	N. C.	—	60	—	—	20	—	N. C.

Same Normal Plasma (Latent Period 180 sec.) was used in cases VI, VII, and IX.

Figures below NORMAL and CARRIER, indicate the concentration of Normal or carrier plasma in the mixture.

N. C. means "No Contraction".

Each Carrier plasma corresponds to a different case.

For the carrier hemophilic mixtures, the 1/10 to 1/40 concentrations were selected. If the 1/10 mixture did not normalize the hemophilic plasma, no more diluted mixtures were studied.

Only female toads were used for these experiments.

The results are registered in table I.

Only women who had hemophilic sons and hemophilic brothers have been included in this table.

The incapacity of a carrier woman to normalize the vasopressin test of an hemophiliac is clear.

Importance of performing the carrier compensation test with plasma from relatives.

The results are registered in table II.

Effect of diluted plasmas on the vasopressin test.

1.5 ml of normal, carrier, and hemophilic plasmas diluted to 25 %

TABLE II

Compensation Vasopressin test in a carrier case tried with three different hemophilic plasmas, two of them being her children (I & II). The three hemophiliacs belonged to type A. Only fresh plasmas were used.

Carrier	Plasma	Concentration	Latent period (sec.) 1/10	1/20	1/40
Hemophilic			No Contraction		No Contraction
I	+	Carrier I (Mother of I & II)			
II	+	Carrier I	20		No Contraction
III	+	Carrier I			20
III	+	Carrier III (Mother Hemophiliac III)		360	No Contraction

The test of Biggs & Douglas served to classify the type of Hemophilia (1).

with saline, were injected in toads, and the vasopressin test tried, as has been described.

The results are registered in table III.

Differences are striking enough to draw attention to the table.

TABLE III

Influence of Dilution of Plasma on the Vasopressin Test

Cases	Latent period (Sec.) Concentration of plasma 100 %	25 %
Normal		
I	180	25
II	20	20
III	15	20
IV	12	10
V	25	20
VI	20	15
VII	35	25
VIII	25	20
IX	30	35
Hemophiliac		
I	No Contraction	No Contraction
II	" "	" "
III	" "	" "
IV	" "	240
V	690	600
Carrier		
I	150	600
II	15	No Contraction
III	25	" "
IV	20	" "
V	50	" "
VI	15	" "
VII *	15	25
VIII	20	No Contraction
IX	15	" "
X	20	" "

* This case is the same considered in table II, mother of two hemophiliacs (I & II), who did not normalize their compensation tests but normalized the test of a hemophiliac unrelated to her family.

Discussion

The incapacity of plasmas from hemophilic-carriers to normalize the vasopressin test of hemophiliac is clear. It might represent a method to differentiate the carriers from those who are not. Sometimes it seems important to perform the test with the plasma of a relative (table II).

Results observed in table II led us to consider that possibly there are other differences among hemophiliacs of the same type, not only quantitative, but qualitative, too.

As normal, hemophilic and carrier plasmas also present important differences when diluted injections of each plasma are made separately, dilution to 25 % with saline, appears as a convenient simplification of the test for most cases.

The interpretation of experiments included in another article ([2]), can be improved with the experiments of this paper. The possibility that an inhibitor in the hemophilic plasma is responsible for the non contraction of glomerules, cannot be sustained. As a matter of fact, the destruction of an inhibitor during storage for the normalization of the vasopressin test performed with stored hemophilic plasma, was a satisfactory explanation.

But an inhibitor cannot be more concentrated in the 25 % diluted plasma than in the non-diluted plasma. The results obtained with the carrier diluted plasmas are clear.

As it was suggested in another article, the effect obtained with the hemophilic plasma appears as the result of the injection of an unbalanced plasma and not of a plasma with a lacking factor. The injection of diluted carrier plasma might represent an unbalanced system. The final relation between albumins and globulins in the blood of the toad, might not be the same with pure and with diluted injected plasma.

Summary

1 — A method is described to diagnose the hemophilic carrier condition.

2 — The Vasopressin compensation test does not normalize by mixing hemophilic and carrier plasma as it is always observed by mixing hemophilic and normal plasma.

3 — In most cases dilution of carrier plasma to 25 % with saline transforms a normal test in a hemophilic one, never observed with normal plasma.

4 — The meaning of these findings is discussed.

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INFLUENCE OF SEVERAL GLUCIDIC METABOLITES ON THE AEROBIC PRODUCTION OF LACTIC ACID BY RAT LIVER SLICES * **

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IT HAS been demonstrated that glucose does not increase the basal oxygen consumption by rat liver slices, whilst several phosphorylated hexoses and fructose increase it from ten to twenty per cent (Niemeyer, 1951; Niemeyer et al., 1953; Niemeyer and Figueroa, 1956). In this paper the aerobic production of lactic acid in the presence of the same substrates has been measured as an index of the catabolism of the particular substrate.

EXPERIMENTAL

Male rats weighing from 200 to 250 g were used. After stunning and exsanguination of the animals, the livers were rapidly excised and placed in cold saline. Liver slices were prepared free-hand with a razor blade and kept in oxygenated cold Krebs solution (modified to contain less calcium) (Krebs, 1933). About 100 mg of liver slices were put in conventional Warburg vessels, to which 2 ml of Krebs-ouffer and the corresponding substrates had been added. The following substrates were assayed: glucose, fructose (S. A. F. Hoffman La Roche & Co. Ltd.), Glucose-6-phosphate (***), Fructose-1,6-diphosphate (***), sodium pyruvate (all from Schwarz Laboratories, Inc.).

The substrates were added to the saline, replacing appropriate amounts of NaCl by equiosmolar solutions of the substances, to obtain the desired concentration. After ten minutes of thermal equilibrium, one

* Aided by a grant from the Williams-Waterman Fund of the Research Corporation, New York.

** The data of this paper were presented at the Sociedad de Biología de Santiago, 1955.

*** G-6-P will designate glucose-6-phosphate and F-di-P, fructose-1,6-di-phosphate.

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vessel from the triplicate of each experimental condition was removed and the lactic acid from the medium and the tissue initial glycogen were determined. After sixty minutes of incubation, lactic acid was measured in the other vessels. The procedure of Walaas and Walaas (1950) with Somogyi (1945) and Nelson (1944) reagents was used for glycogen and the method of Barker and Summerson (1941), modified by Hullin and Noble (1953) for lactic acid.

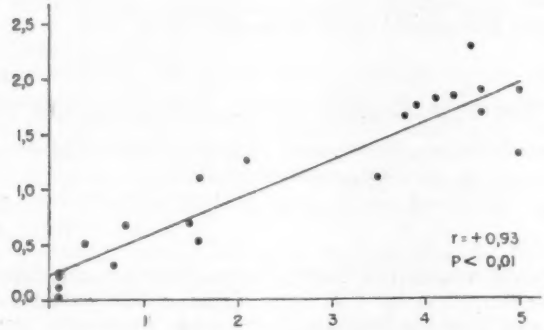

FIG. 1. — *Lactic acid production by rat liver slices with variable glycogen content. No added substrates. Abscissa, initial glycogen content in grams %; ordinate, lactic acid production in µM/100 mg fresh weight/hr.*

RESULTS

Lactic acid production without substrate. — Liver is characterized by its low production of lactic acid under aerobic conditions (Warburg, 1928). Our average figures are very similar to those of other investigators, but ours show a higher variability, ranging from 0.1 to 2.3 µM per 100 mg tissue per hour. This is explained by the fact that the initial glycogen content of the slices is very different from one experiment to another and, as it is shown in Fig. 1, there is a direct proportionality between the aerobic production of lactic acid and the glycogen content of the liver ($r = 0.93$; $P < 0.001$).

This influence of glycogen content had been observed by Craig (1943) and by Olson (1951), who compared the aerobic formation of lactic acid by liver slices from fasted and normal fed rats. The dependence of anaerobic glycolysis on the glycogen content of the slices had already been established by several authors (Rosenthal, 1929, 1930; Orr and Stickland, 1941; Warren and Ebaugh, 1946).

Effect of glucose and fructose. — The addition of glucose to the incubation medium produces a significant increase in the formation of lactic acid (Table I). These results differ from those of many investigators, who have concluded that the change of aerobic glycolysis induced by the presence of glucose is not significant (Dickens and Greville, 1933;

TABLE I

Extraproduction of lactic acid by rat liver slices induced by the addition of different substrates to the incubation medium

Substrates (30 mM/l)	*Nº of experiments*	*Extraproduction of lactic acid**
Glucose	32	0.62 ± 0.06
Glucose-6-phosphate	19	1.08 ± 0.16
Fructose	8	1.88 ± 0.42
Fructose-1,6-diphosphate	5	3.22 ± 0.32

* Arithmetic mean and standard error of the extraproduction of lactic acid in µM/100 mg fresh weight/hr induced by the presence of substrates.

Elliot, Greig and Benoy, 1937; Orr and Stickland, 1941; Warren and Ebaugh, 1946; Olson, 1951). The discrepancy may be due to the concentration of glucose used. In fact, Fig. 2 shows that the increase in lactic acid formation is evident only when glucose concentration is over 20 mM/l, and the majority of the authors have used 10 mM/l.

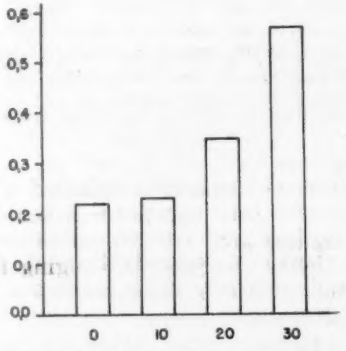

FIG. 2. — *Effect of glucose concentration on the production of lactic acid by rat liver slices. Abscissa, glucose concentration, in mM/l; ordinate, lactic acid production in µM/100 mg fresh weight/hr.*

Fructose enhances the formation of lactic acid much more than glucose (Table I). According to our best information this effect of fructose has not been investigated under aerobic conditions, but only in anaerobiosis (Rosenthal, 1929; Dickens and Greville, 1932).

Glycogen content of the liver slices does not significantly affect the extraproduction of lactic acid from glucose ($r = 0.17$; $P > 0.8$) or from fructose ($r = -0.60$; $P > 0.1$).

Influence of some phosphorylated hexoses. — G-6-P increases lactic acid production more than glucose (Table I). Statistical analysis of paired

experiments reveals that the average difference between these two substrates is 0.46 ± 0.10 μM per 100 mg per hour ($P < 0.01$). The largest lactic acid extraproduction was obtained when F-di-P was used (Table I). The correspondent figures differ significantly from those observed with fructose in paired experiments ($P < 0.05$).

We have not found data in the literature concerning the influence of hexosephosphates on the aerobic formation of lactic acid by liver slices. Rosenthal (1930) has published a few experiments performed under anaerobic conditions.

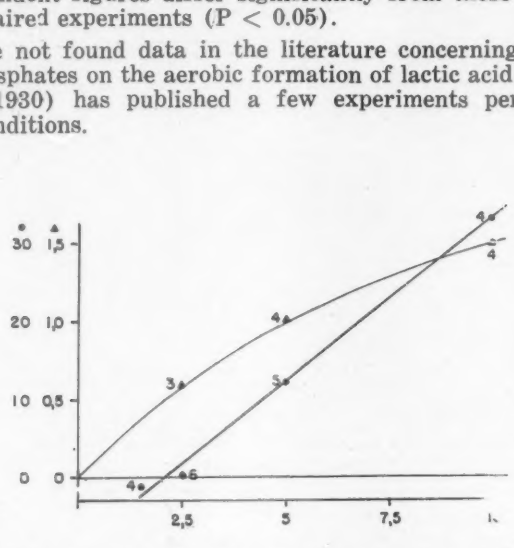

FIG. 3. — *Influence of pyruvate concentration on the oxygen uptake and the extra production of lactic acid by rat liver slices. Abscissa, pyruvate concentration, in mM/l; ordinate, change in oxygen uptake in per cent over the basal (●) and lactic acid extraproduction in μM/100 mg fresh weight/hr (△).*

Effect of pyruvate. — The results obtained with pyruvate are represented in Fig. 3. Diverse concentrations of the substrate were assayed and lactic acid production and oxygen uptake were measured. It was interesting to learn that at a concentration of 2.5 mM/l or less, the oxygen uptake was not modified, whilst lactic acid production was clearly increased.

DISCUSSION

The results obtained without added substrate confirm and extend the observation that the rate of glycogen degradation, as measured by the formation of lactic acid, is a function of its concentration within the hepatic cells. This permits to explain the influence of glycogen content on several metabolic reactions of liver slices (Rosenthal, 1929; Craig, 1943; Niemeyer and Figueroa, 1955; Niemeyer, Figueroa and González, 1956).

Our results show that extraproduction of lactic acid from G-6-P is higher than that from glucose and point out that the conversion of glucose to G-6-P is a limitating stage in liver metabolism. This finding is in full accordance with the results obtained in the study of oxygen

uptake under equal experimental conditions (Niemeyer, 1951; Niemeyer et al., 1953; Niemeyer and Figueroa, 1956) and enable us to understand why the hepatic cell has a limited capacity for glucose degradation. Our data also show that F-di-P induces a much greater extraproduction of lactic acid by liver slices than G-6-P. This had already been observed by Olson, Robson, Richards and Hirsch (1950) in rat liver homogenates submitted to anaerobic conditions in ATP-enriched media. Thus, it must be granted that there is another limitating stage, namely the conversion of hexosemonophosphates to F-di-P, a fact which is also evident in studies on oxygen uptake. This difference in lactic acid extraproduction may also be explained by supposing that while the degradation of hexosemonophosphates follows the oxidative pathway predominantly, that of F-di-P may proceed mainly along the glycolytic route.

Notwithstanding it must be pointed out that the medium where liver slices have been incubated can produce lactic acid from F-di-P, but not from the other substrates assayed. This indicates that some enzymatic systems may diffuse out of the liver cells, as has been shown with diaphragm (Zierler, Levy and Andres, 1953).

The results indicate that the production of lactic acid from fructose is far higher than that observed in the presence of glucose or G-6-P. Niemeyer and Figueroa (1956) have shown that fructose is a better precursor of glycogen than glucose and several hexose-phosphates. Thus, it can be deduced that fructose is not acting after its conversion to free glucose or to glucose-phosphates. It is possible then that fructose disposes of two completely different pathways for its metabolism in the liver: the formation of glycogen on one hand and the conversion into triose on the other. This assumption is supported by the observation of Renold, Hastings and Nesbett (1954) who have shown that in liver slices from alloxanic rats the glycogenesis from fructose is inhibited, but its degradation to carbon dioxide is not impaired.

It is rather interesting that glucose increases the production of lactic acid, although it does not modify the oxygen consumption. In order to explain this fact, it may be assumed that the reduction of pyruvate to lactate is easier than its oxidation to CO_2. Therefore, with low concentration of pyruvate inside the cells only the production of lactic acid may be noted. In fact, with a concentration of pyruvate as low as 2.5 mM/l, it was observed that while the oxygen consumption did not change, the production of lactic acid increased to the same level obtained with glucose at concentrations twelve times higher. This suggests that the degradation of glucose produces a concentration of pyruvate into the cell similar to that attained by adding a small quantity of this metabolite to the medium. It is worthwhile to recall that it has been demonstrated with labeled pyruvate that the production of lactate in rat liver slices is approximately equal or twice that of $C^{14}O_2$ (Olson, 1951; Hastings, Teng, Nesbett and Sinex, 1952).

Summary

The aerobic production of lactic acid by rat liver slices under several experimental conditions has been studied.

The formation of lactic acid by the slices incubated without substrates depends on the initial glycogen content of the tissue.

The extraproduction of lactic acid induced by the addition of different substrates (30 mM/l) varies with the substrate employed, in the following order: F-di-P > Fructose > G-6-P > glucose.

The addition of pyruvate at a concentration of 2.5 mM/l, while not increases the oxygen consumption of the liver slices, augments the production of lactic acid.

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PROCEEDINGS OF THE ARGENTINE SOCIETY OF BIOLOGY

Buenos Aires, August 2nd, 1956

Reinforced effect of insulin due to hypoglycemiant sulphonamides. B. A. HOUSSAY AND R. H. MIGLIORINI. *Instituto de Fisiología, Facultad de Medicina, Buenos Aires.*

1) Sulphanilyl-n-butyl urea (Bz 55) has no hypoglycemic action in totally pancreatectomized dogs, under chloralose anesthesia or non-anesthetized.

2) This substance markedly increases the hypoglycemic action of an infusion of insulin (0.04 U per Kg. per hour) during 3 hours. The fall in the blood sugar is greater and prolonged during many hours.

Sensitiveness of adrenalectomized animals to hypoglycemiant sulphonamides. B. A. HOUSSAY, J. C. PENHOS, R. H. MIGLIORINI Y J. R. BOWKETT. *Instituto de Biología y Medicina Experimental, Buenos Aires.*

1) Three sulphonamides (Bz 55, D 860, 2254 RP) have a hypoglycemic action in dogs, rats and toads.

2) The blood sugar fall is very rapid and intense in the absence of adrenals in animals of the three species. Death occurs in convulsions in 2-6 hours in adrenalectomized dogs or rats injected 200 mg/Kg. intravenously.

3) After pancreatectomy hypoglycemia and death are not provoked by these sulphonamides in adrenalectomized dogs.

4) Cortisol and cortisone have a protective action on hypoglycemia or death in the adrenalectomized animals injected with sulphonamides.

5) Injection of glucose, in high and repeated doses, corrects the hypoglycemia and convulsions and prevents death in the adrenalectomized dogs or rats injected with sulphonamides.

Testosterone and adrenal gland of the toad. C. E. RAPELA AND M. F. GORDON, *Instituto de Biología y Medicina Experimental, Buenos Aires.*

Testosterone propionate was injected in the dorsal lymphatic sac of the male toad, *Bufo arenarum* Hensel, in order to study its effect on the adrenalin and noradrenalin content, on the weight of the adrenal glands and on the histological picture of the corticoadrenal cells. Tricloroacetic acid (5 %) extracts and colorimetric determination of adrenalin and noradrenalin (Euler and Hamberg, 1949) were used.

1º) Testosterone 1mg/day, during 21 days induced a significant increase of the porcentage and of the absolute amount of adrenaline when the toads were injected during the months preceeding sexual activity, July and August (Winter). In two out of three experiments a significant decrease was observed when injected at the end of September, January and April.

2º) An increase of the weight of the adrenals ($+ 3.33 \pm 1.2$ mg. $P < 0.05$) and a hypertrophy of its cortical cells occurred when the toads were injected with 1 mg/day during 21 days.

3º) When the normal animals were injected with 1 mg/day during 4 days the noradrenalin content of the adrenals was higher than in the non treated animals. The increase was not statistically significant.

4/) In the hypophysectomized toads injected 1 mg/day during 4 days, was observed. In the hypophysectomized animals in which the last injection was given 6 hours previous to the autopsy, the adrenal weight decreased significantly.

The changes observed in the adrenal weight cannot be accounted for differences in body weight.

Effect of hypertensin and renin on diuresis. J. V. URANGA. *Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires.*

Hypertensin and renin, injected intravenously in the rat, produced in most of the cases a brief initial inhibition of urinary flow, followed by a sharp increase in the diuresis.

The similarity found between the effects of both these substances would lead us to think that the action of renin on diuresis is exerted thrcugh hypertensin.

Buenos Aires, September 6th, 1956.

Tumors of testicular graft in the spleen of the castrated rat. E. FELS AND G. E. BUR. *Instituto de Maternidad, Dirección Nacional de Asistencia Social, Buenos Aires.*

Seven tumours have been obtained in 30 rats observed for more than 270 days after intraesplenic testicular grafts (23,3 %). Microscopically, typical adenomas of Leydig cells were observed, that have similar aspect to adenomas originating in intraesplenic ovarian grafts. These tumours would appear to possess estrogenic activity, lacking androgenic function.

Effect of hydrocortisone infusion on the blood pressure of adrenalectomized dogs. H. E. J. HOUSSAY. *Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires.*

1) The intravenous infusion of hydrocortisone produced a slight increase in the blood pressure of dogs 48 hours after adrenalectomy had been performed.
2) A commercial sample of a hydrocortisone solution produced a toxic hypotension in these animals.
3) Normal dogs did not show the increase in blood pressure observed in the adrenalctomized.
4) After hydrocortisone infusion, the response to a sudden injection of noradrenaline was diminished both in adrenalectomized and in normal dogs.

Effect of Cortisone or ACTH in rats treated with Aminopterin of Folic Acid.

J. C. PENHOS AND R. NALLAR: *(Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires).*

Experiments were carried out in prepuberal male rats, weighing 55 to 85g and treated with ACTH (2 mg/day/rat) or cortisone (0.5 mg/twice a day/rat) and folic acid (1 mg/day/rat) or aminopterin (0.01 mg/day/rat) during 8 days, observing:

1) the normal increase in body weight was inhibited by the action of ACTH and cortisone. This inhibitory effect was significatly reinforced by aminopterin and not modified by folic-acid.

2) the weight of the thymus and the spleen was decreased by the effect of ACTH and cortisone. This effect was significantly reinforced by aminopterin and not modified by folic acid.

3) the weight of the adrenals was increased by the effect of ACTH, this effect not being modified by folic acid or aminopterin. The weight decrease produced by cortisone was inhibited by folic acid and aminopterin.

4) the characteristic effects of ACTH and cortisone were reinforced by aminopterin, except the action of cortisone on the adrenals.

Effect of gamma-globulin of normal and cancer human serum on the chick embryo. E. SACERDOTE DE LUSTIG AND B. FISZER. *Institute of Oncology and Bacteriological Institute "Malbran", Buenos Aires.*

One series of chick embryos, 9-11 days of incubation have been inoculated intravenously, with total serum, fraction IV- V- VI and Gammaglobulinic of 10 Cohn Tecnique of healthy blood donors, of benign tumour carrier's blood, and another series with blood of histologically malignant tumour carriers, of Hodgkin patients, of tumour carriers who have been operated, irradiated and treated. 4- 6 days after the inoculation we have studied the incidence of monstrous embryos among those surviving, and we have observed the following:

1) Normal serums produce 1.2 % monstrosities, the gamma-globulin, 1.3 %.
2) Benign tumor serums produce 1 % of the monstrosities.
3) Operated, irradiated or treated tumours produce 1 % of the monstrosities.
4) The Cancerous fraction IV-V-VI produces 1.4 % of monstrosities.
5) Total cancerous serums produces 52 % and gamma globulin 65 % of monsters.
6) The gamma fraction repeats the malformations of the total serum with greater incidence, perhaps there contain some sort of enzyme or virus factor, wich acts on the embryo. This factor mantains its activity at -20° and is destroyed a 60°.

The most frequently observed malformations, are of a general type and appear in the form of total edema of the embryo or confined to the occipital region. Sometimes inhibitions are observed in the growth; sometimes hemorrhages, anemia or the absence of feathers.

The local malformations include either the bone system (brachimelia, hemimelia, absence of parietal occipital and jaw bones) or the central nervous system (excerebration, microcephalia, acephalia); or the eyes (total or partial anofthalmia, reabsorbtion of ocular globe or the beak (crossed over, parrot beak, brachignatia) and repeat all the monstrosities produced by physical-chemical agents or by virus.

7) A relation between the histological type of tumor and the embryo reaction cannot be established.

The most indifferentiated tumours and without any formed treatment, are the ones which have shown the greater incidence.

8) A relation between the degree of evolution of the tumour and the degree of monstrosity, has not been established.
9) The relation between the site of the monstrosity and that of the tumour, cannot be established, because of the malformations of the bone system appear more often in breast and lung cancer; anogthalmia and acephalia in tongue cancer, but there are many clinical factors, hormonal, evolution of the tumour carrier and others in the receiving embryo, which act together, and vary the incidence of the monster process.

Only the quantitive statistical study of the plasmatic proteins of many carriers of the same histological type, with the same degree of evolution, of the one organ on genetically pure embryos, will, from a diagnostic point of view, be able to give us an idea of the value of this investigation in the future.

We are now studying if an immunising anti-cancerous serum can act as an antagonism of the teratogenous action of the cancer serum.

PROCEEDINGS OF THE SOCIETY OF BIOLOGY OF SANTIAGO (CHILE)

April 24 th, 1956.

Effect of cortisone on experimental infections produced by *Tripanosoma cruzi*. T. PIZZI AND J. CHEMKE. *Departamento de Parasitología, Universidad de Chile.*

The authors made a study to investigate the mechanisms by which cortisone increases the severity of *Trypanosoma cruzi* infections in rats. A highly virulent and standardized strain of the parasite and inbred rats of the A/C strain were used. Two different doses of the hormone were administered from the onset of the infection (with appropriate controls) and serial analysis of the tissue changes, parasitemias and antibody titers were performed.

The main results may be summarized as follows:

1. — High doses of cortisone (5 mg per 60 g rat weight) intensified the infection. The animals that received the hormone developed an acute disease, with rapidly increasing parasitemias and death within 14 days, whereas all the controls survived indefinitely after two rises in the parasitemia figures, each one followed by a parasitic crisis.

2. — Histopathologically, a more intensive parasitic invasion of the tissues could be observed, as well as a less intensive inflammatory reaction and more pronounced involutions in the lymphatic organs of cortisone-treated animals, as compared to the controls. The phagocytic activity of the macrophages was markedly lowered by cortisone.

3. — The developmental rate of reproduction of the parasites was significantly accelerated in the animals treated with high doses of the hormone.

4. — A marked decrease in antibody titers was observed in the cortisone-treated animals as compared with the controls.

5. — Summarizing, the aggravating action of cortisone in these experiments can be explained by the following interacting factors: a) acceleration of the reproductive cycle of the parasite; b) inhibition of the defensive inflammatory reaction; c) decrease of the phagocytic capacity of the inflammatory macrophages and d) lowering of the titers of "non lytic" antibodies that play a role in immunity.

6. — Smaller doses of cortisone (below 1 mg) did not change the course of the infection, although the appearance of the "non lytic" antibodies was delayed and their titer lowered.

Theory of Biological Similarity applied to some data of comparative physiology. B. GÜNTHER AND E. GUERRA. *Instituto de Fisiología, Escuela de Medicina, Universidad de Chile and Instituto de Matemáticas, Universidad de Concepción, Chile.*

May 8th, 1956

Bioelectric potentials of the neuromuscular spindle in the toad (*). C. EYZAGUIRRE AND J. VIAL. *Laboratorio de Neurofisiología and Departamento de Anatomía, Universidad Católica de Chile.*

The afferent discharges of the neuromuscular spindle are modulated by contraction of the intrafusal muscle fibres. Contraction is elicited in the frog by activation

(*) This work has been financed in part by grants from the Rockefeller Foundation and the Fundación Gildemeister.

of large diameter motor axons (Katz, B., *J. exp. Biol.*, *26* : 201, 1949) while in the cat it occurs after stimulation of the small-nerve motor fibres (Kuffler, S. W., Hunt, C. C. and Quillian, J. P., *J. Neurophysiol.*, *14* : 29, 1951).

This study was designed to obtain direct evidence on the behaviour of the intrafusal fibres in amphibians by the micrcelectrode technique, employing micropipettes filled with 3M KCl having a tip of about 0.5, and a DC resistance of 10 to 60 megaohms. The preparation was the M. extensor longus digiti IV of the Chilean toad (Callyptocephalus gayi). The muscle was dissected out and placed in a lucite dish filled with Ringer solution. The nerve was lifted out of the saline for stimulation and recording with fine platinum-iridium electrodes. After suitable curarization (1-2 umol d-tubocurarine HCl/1t) the extrafusal junctions were blocked but the spindles remained contracting apparently unimpaired. The intrafusal fibres were pierced by the microelectrode and resting membrane potentials of 61.3 mV to 88.6 mV recorded. A single nerve shock produced action potentials of 84 mV to 119.3 mV and always overshooting the zero baseline by 12.5 mV to 31.6 mV.

It is concluded that the intrafusal muscle bundles are formed by twitch fibres, although the presence of some "slow" intrafusal fibres has not been excluded in these experiments.

May 22th, 1956

Cortisone and metastases of intrasplenic ovarian tumours in two strains of mice. E. MARDONES AND A. LIPSCHUTZ. *Instituto de Medicina Experimental. Servicio Nacional de Salud.*

Cortisone stimulates the metastatic spread of a transplanted non-metastasizing adenocarcinoma (Agosin, M., Christen, R., Badínez, O., Gasic, G., Neghme, A., Pizarro, O. and Jarpa, A., *Proc. Soc. exp. Biol. & Med.*, 80 : 128, 1952). This finding of the Chilean group has been corroborated with other transplantable tumours though not with all (for references see Martínez and Bittner, *Proc. Soc. exp. Biol. Med.*, 89 : 569, 1955). Will cortisone stimulate the metastatic spread of a tumour originating in the body due to a hormonal imbalance? Two strains of mice were used: C57b1 and BalbA. In C57b1 without cortisone so far no metastases has been found: in BalbA without cortisone metastases appeared in 4 animals as late as 404 to 570 days after grafting the ovary. In the present work cortisone was administered through absorption from pellets implanted beneath the skin ab. 6 months after grafting the ovary into the spleen. There were no metastases among 55 animals of the two strains without cortisone which had tumours and were killed 10 to 13 months after grafting the ovary. Neither were metastases found in 37 animals with cortisone which had tumours. Quantities of cortisone absorbed per day were, indeed, smaller than in the experiments of Agosin et al. However, they were sufficient to influence considerably the evolution of the intrasplenic ovarian tumour (see next communication).

Steroid homeostasis and tumorigenesis - New experimental aspects. E. MARDONES AND A. LIPSCHUTZ. *Instituto de Medicina Experimental. Servicio Nacional de Salud.*

Cortisone reduced the incidence of the intrasplenic ovarian tumour in both strains of mice used (C57bl; BalbA). More notable were other results with cortisone such as: 1) degenerative phenomena in the tumourous graft, and 2) change of the prevalent type of the ovarian tumour. In C57bl without cortisone only 20 to 25 per cent of the growths were luteomata and the remaining growths were granulosa-cell tumours. Under the influence of cortisone the percentage of animals with luteomata, including animals with degenerated luteomatous tissue, and animals with nodules of luteinized cells not classified as tumourous, increased to 80 per cent of the ovarian grafts in the cortisone animals were similar to the slowly growing intrasplenic ovarian growths in guinea-pigs in the first two years after grafting. The following tentative suggestions were made: 1) that the antitumorigenic action of cortisone was by interference

in the gonadotrophic activity of the hypophysis; and 2) that differences as to the evolution of intrasplenic ovarian tumours between guinea-pigs and mice (or between strains of mice) might possibly be explained by a genetically given difference in the pattern of steroid homeostasis.

Studies on the metabolism of Echinococcus granulosus. I. General chemical composition and respiratory reactions. M. AGOSIN, T. VON BRAND, G. F. RIVERA AND P. MCMAHON. *Departamento de Parasitología, Universidad de Chile, and U. S. Department of Health, Education and Welfare. Public Health Service. National Institute of Health. National Institute of Allergy and Infectious Diseases. Bethesda, Maryland.*

Chemical determinations in hydatid cyst scolices indicate the presence of large amounts of protein and smaller amounts of lipids. Less inorganic substances are present than in other immature tapeworms. The scolices store two polysaccharides: glycogen and a polysaccharide containing galactose and glucosamine.

The rate of oxygen uptake is fairly rapid, with an over-all RQ of 0.88. There is also a pronounced anaerobic CO_2 production, but the source of this CO_2 has not been established.

The Q_{10} of the oxygen consumption is 2.1 in the range 28 to 38°C; temperatures of 42 and 45°C are beyond the optimum. The respiration of hydatid cyst scolices remains constant in the pH range 4.5 to 8.5, but the ionic composition of the medium has a considerable influence on the respiratory rate, hydatid cyst fluid and Ringer's solution sustaining the respiration best.

The aerobic and anaerobic gaseous exchanges are highly sensitive to glycolysis inhibitors, but not to *dl*, glyceraldehyde. The aerobic respiration is markedly cyanide sensitive and is also inhibited by fluoracetate, but not by malonate.

June 5th, 1956

Reinnervation of the nictitating membrane by cholinergic fibers (*). C. VERA, J. VIAL AND J. V. LUCO. *Departamento de Anatomía and Laboratorio de Neurofisiología. Universidad Católica de Chile.*

In eight adult cats, the central stump of the hypoglossal nerve was sutured to the post-ganglionic branches of the superior cervical ganglion, after excision of the latter. After periods of time that varied from 60 to 150 days, the reinnervation of the nictitating membrane was studied.

Under these circumstances, stimulation of the hypoglossal nerve result in contraction of the nictitating membrane. This effect is abolished by small doses of atropine, but is unaffected by curare or Dibenamine.

The fatigability of this new neuromuscular preparation is similar to that of a somatomotor or preganglionic synapse. The maximal response is obtained at a frequency of stimulation of 60 per second, which makes it differ from most neuroeffector preparations.

The hypersensitivity to adrenaline of the denervated nictitating membrane disappears after reinnervation by fibers of the hypoglossal nerve.

The hypoglossal fibers that invade the peripheral stump of the post-ganglionic sympathetic develop a fairly thick myelin sheath. When the invading fibers come into contact with the smooth muscle of the membrane, they lose their myelin and build up a terminal plexus, which is morphologically very similar to the normal sympathetic end-plexus.

This work was assisted by gants from the Fundación Cildemeister and the Rockefeller Fondation.

The influence of lactic acid on the synthesis of desoxiribonucleic acid by slices of rat liver. M. PLAZA DE LOS REYES, J. MARTENS AND J. ARAYA. *Instituto de Química fisiológica y patológica. Escuela de Medicina. Universidad de Chile.*

The incubation during 4 hours of slices of rat liver in an oxygenated medium supplemented by 0.2 % Ribonucleic acid (R.N.) gives place to a net synthesis of Des-

oxiribonucleic acid (D.N.) (Cruz-Coke, E., Plaza de los Reyes, M., Bradford, I., Pómez, R. and Araya J., *Bol., Soc. Biol. Stgo. (Chile)*, 11-II-1954). This synthesis is inhibited by KCN and I_2 (Cruz-Coke, E., Plaza de los Reyes, M., Martens, J., Matus, I. and Araya, J. in press) but not by dinitrophenol. When the liver slices are obtained from rats previously treated with thiouracil for a period of time enough to produce hypothyroidism, the slices lose their ability to synthesize D.N. acid. It would be interesting to know the influence of substances that during their metabolism restore ATP. With this purpose sodium lactate was tested and the results are summarized in this paper.

Twenty eight adult male and female rats were killed by decapitation. Liver slices were obtained, and groups of slices (each group of 200 mg) were suspended in borate buffer at pH 8, with the addition of 0.2 % R. N. acid.

The 28 animals were paired in 14 experiments, and the slices were incubated with and without the addition of 3×10^{-2} M sodium lactate. The incubation was performed during 4 hours in an oxygenated medium, under continuous shaking of the flasks. Nucleic acids were obtained by the Schneider technique (Schneider, W. C., *J. biol. Chem., 161* : 293, 1945) and D.N. acid was measured by the Stumpf method (Stumpf, P. K., *J. biol. Chem. 169* : 267. 1947).

Results are presented in Table I where the two series are statistically compared. The increment of D.N. acid in the control group, after 4 hours incubation was 0.264 mg/g and in lactate treated group it was 0.544 mg/g. The average difference between both rises is 0.280 mg/g, and it is significantly different from 0 ($t = 4.35$) which represents the lack of difference between both series.

TABLE I

Synthesis of desoxiribonucleic acid by rat liver slices, with and without 3×10^{-2} sodium lactate

Desoxiribonucleic acid (mg/g liver slices)				Increment of D.N. after 4 hours		Difference
Control		With Na lactate		Control	With lactate	
0 hrs	4 hrs	0 hrs	4 hrs			
1.786	2.051	1.745	2.288	0.264	0.544	0.280 ± 0.064
$t = 2.8$		$t = 14.3$			$t = 4.35$	

(Each figure represents the average of 14 experiments)

The favourable effect of sodium lactate on the synthesis of D.N. acid is in line with the inhibition of this synthesis by cyanide. These facts demonstrate that this synthesis consumes energy and the lactate effect must be interpreted as energy apported for the resynthesis of ATP.

In conclusion it may be said that slices of rat liver, incubated with 3×10^{-2} M. sodium lactate, increase significantly their content in D. N. acid., as compared with those incubated without lactate.

June 26th, 1956

Electrogram changes induced by lack of metabolites on the isolated guinea pig heart. M. PENNA, A. ILLANES, J. RIVERA AND J. MARDONES. *Laboratorio de Farmocología, Escuela de Medicina. Universidad de Chile.*

The electrogram changes of isolated guinea pig hearts perfused with Tyrode solution with or without glucose were studied.

On the hearts perfused without glucose, the following changes were registered: lengthening of the P-R interval, A-V dissociation, decrease of auricular and ventricular depolarization voltages and ventricular fibrillation. The perfusion with Tyrode solution containing glucose reversed the ventricular fibrillation to a normal rhythm.

As perfusion progressed, a slight decrease of the auricular rate was observed in

the hearts perfused either with or without glucose; this decrease was of the same order in both conditions.

When perfusing from the start with glucose there was neither significant lengthening of P-R interval, A-V dissociation nor ventricular fibrillation. Auricular and ventricular depolarization voltages decreased slightly, but these changes were significantly delayed in comprarison with those observed in the hearts perfused without glucose.

Electrogram changes induced by malonate and succinate on the isolated guinea pig heart. M. PENNA, J. RIVERA, A. ILLANES AND J. MARDONES. *Laboratorio de Farmacología, Escuela de Medicina. Universidad de Chile.*

The electrogram changes of isolated guinea pig hearts perfused with Tyrode solution with and without glucose and containing sodium malonate or sodium succinate, were registered.

The presence of malonate did not modify the general pattern of changes induced by perfusing without glucose (Penna, M., Illanes, A., Rivera, J. and Mardones, J., *Proc. Soc. Biol. Stgo. (Chile)* in press) but the ventricular fibriliation was of shorter duration and followed by the absence of electrical activity of the ventricles. Neither the ventricular fibrillation nor the electrical ventricular standstill were reversed by perfusion with Tyrode solution containing glucose.

Perfusion with Tyrode containing glucose (5.5 mM/l) and malonate (3.3 mM/l) induced similar changes than perfusion with Tyrode without glucose. Ventricular fibrillation started later with Tyrode with glucose and malonate than with Tyrode without glucose and with malonate.

Perfusion with Tyrode solution without glucose and containing sodium succinate (3.4 mM/l) generally induced the same changes than perfusion with the same solution without succinate. However, no decrease in the auricular rate was observed in the presence of succinate.

Fibrinolytic paracoagulation with different substances. M. DERECHIN AND S. SZUCHET (*). *Laboratorio de Química; Escuela Dental. Universidad de Chile.*

Fibrin and fibrinogen lysed by plasmin may be clotted by protamine sulphate or toluidine blue (Derechin. M., *La Prensa méd. Arg., 40* - 2763. 1953). In this work it was found that many other substances have same effect (table I). Lysed fibrin Lysed fibrinogen requires, for clotting, the previous attack of thrombin (Fibrinogen reclots by the simple addition of these drugs (Fibrin Fibrinolytic Paracoagulation). Fibrinolytic Paracoagulation) In both cases paracoagulation is instantaneous.

In this paper it is assumed that forces which determine polymerization of native activated fibrinogen participate in the paracoagulation reaction and that fibrinogen, incoagulable by thrombin after plasmin action, may still be activated by thrombin (at least during the paracoagulation period).

TABLE I

Substances	Minimal active concentration for a solid clot (mM/l)
Anyline	3
Glycine	10^3
Mercuric chloride	3×10^{-1}
p-Nitrophenol	4×10^{-1}
Nynhidrine	3
Protamine sulphate	5×10^{-3}
Sodium chloride	5×10^3
Sodium sulphate	4×10^2
Tyramine	1
Zinc suphate	2×10^{-2}
Distilled water	dilution to 66 % of lysed fibrin or of lysed-activated fibrinogen causes paracoagulation.

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July 10th, 1956

Influence of the thyroid hormone on adrenal gland activity. E. DUBROCK, C. GRADO AND J. TALESNIK. *Instituto de Fisiología and Laboratorio de Fisiopatología. Escuela de Medicina. Universidad de Chile.*

Some mechanisms involved in the modification of adrenal activity due to lack or excess of thyroid gland, were studied in the rat.

a) Thyroidectomy induces adrenal involution and an increase in the gland ascorbic acid concentration. On the other hand, thyroxine administration leads to adrenal hypertrophy and ascorbic acid depletion.

b) Thyroxine given to hypophysectomized animals increases oxygen consumption as in normal conditions but it shortens the rat's survival. This latter effect can be counteracted with cortisone.

c) Relatively high doses of cortisone produces adrenal atrophy and increases ascorbic acid concentration.

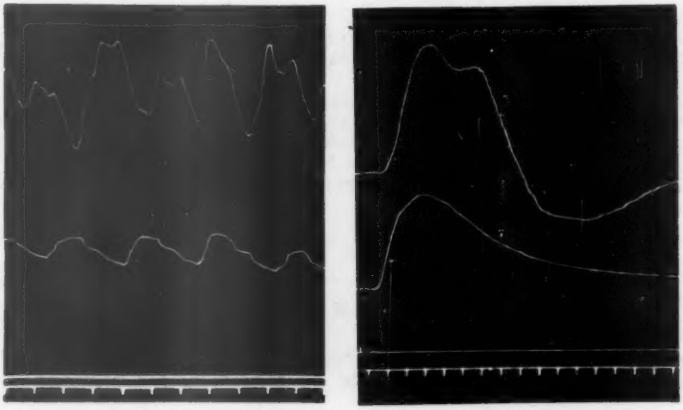
d) Cortisone administered together with thyroxine abolishes the gross morphological adrenal changes induced by the latter.

The results are discussed mainly from the viewpoint of endocrine-interrelationships. Emphasis is placed on the adaptation of the adrenal cortex to conditions of increased corticoid requirements.

Spontaneous activity and contractile responses to adrenaline of denervated auricular muscles. J. V. LUCO AND P. SANCHEZ. *Laboratorio de Neurofisiología. Universidad Católica de Chile.*

Spontaneous activity. In most of the cats with facial nerves severed from 6 to 13 days before, spontaneous activity of the auricular muscles were recorded. This activity increases after contractile responses evoked by drugs.

Previous studies on denervated facial muscles did not report this spontaneous activity (for references see Cannon & Rosenblueth, 1949. The Supersensitivity of Denervated Structures. The Macmillian Co. New York). The very isotonic conditions used in these experimental series may account for our observation.



Upper record: denervated auricular muscles.
Lower record: denervated nictitating membrane (both for 10 days).
Left figure: Spontaneous activity. Time 1 minute.
Right figure: Contractile response to intravenous of 5 μg of adrenaline. Time: 10 sec.

Contractile response to adrenaline. Intravascular injection of synthetic adrenaline (epinephrine Winthrop) produced contractile responses of the chronically denervated auricular muscles in all experiments. It was never observed in the acutely denervated side. The sensitivity of these muscles to adrenaline was comparable to the chronically denervated nictitating membrane. As shown in the figure, this response lasts for about 3 minutes; acetylcholine responses were also prolonged. Dibenamine (20 mg/Kg, intravenously) blocked the adrenaline response. The removal of the superior cervical ganglion (6-12 days previously) did not affect the adrenaline contractile action.

Euler, US. and Gaddum, J. H. (*J. Physiol., 73* : 54, 1931) reported a contractile effect of adrenaline on denervated facial muscles of the dog and also noted its very prolonged time course. Denervated facial muscles are sensitive to other pressor amines (Luco, J. V., Eyzaguirre, C. and Pérez, F., *J. Pharmacol.* 93 : 261, 1949) and skeletal muscles of the dog's leg also respond to adrenaline (Bulbring, E. and Burn, J. H., *J. Physiol., 86* : 61, 1936).

The great sensitivity of these muscles to adrenaline reopens the discussion on the mechanism of the Rogowicz phenomenon and on the "fright reaction" described by M. S. Bender (*Amer. J. Physiol.*, 121 : 609, 1938).

The spontaneous activity and the very slow contractile response of these effectors suggest that muscular alterations induced by motor nerve section make them behave like smooth muscles or like the "slow muscle system" of amphibians.

Curare-Acetylcholine antagonism in normal and denervated auricular muscles. P. SÁNCHEZ AND J. V. LUCO. *Laboratorio de Neurofisiología. Universidad Católica de Chile.*

The action of denervation on the excitability of auricular muscles to the blocking effect of *d*-tubocurarine on the acetylcholine response, was studied in cats.

It was observed that a dose of curare which did block the dose of acetylcholine necessary to stimulate the normal muscles showed a minor effect on the denervated side, activated by a dose of acetylcholine about a hundred times smaller.

Relation between fibrinolytic paracoagulation and degree of fibrinogen proteolysis by plasmin. S. SZUCHET AND M. DERECHIN (*). *Laboratorio de Química; Escuela Dental. Universidad de Chile.*

Bovin fibrinogen (final concentration 5.150 g/l; 90-96 % clottable protein by thrombin) was incubated with three different plasmin concentrations (pH 7.6; ionic strength 0.2; at 40°C). Increase in non proteic nitrogen, coagulation by thrombin and fibrinolytic paracoagulation were simultaneously studied at intervals.

As it was expected, the time elapsed for the mixture became unclottable by thrombin but it was calculated by interpolation to be equal for the three series and approximately 10×10^{-3} mg N/ml.

The close relationship between N liberation and the behaviour of lysed fibrinogen when tested against thrombin and paracoagulant drugs, enables us to assume that fibrinolytic paracoagulation is closely related to the degree of fibrinogen proteolysis by plasmin and that liberated NPN comes from plasminfibrinogen reaction. The question whether liberated peptides play a passive or an active roll on the clotting of fibrinogen remains to be solved.

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July 24th, 1956

Experimental study on the reflex regulation of glycemia. A. STEINER. *Laboratorio de Fisiopatología. Escuela de Medicina. Universidad de Chile.*

It has been shown in this paper that the level of glucose in the blood can be regulated through a reflex mechanism.

The carotid artery of the dog was sectioned in its cervical part, above the suprasternal fossa and in the thyrode region, but without removing its connections with

In vol. 6, Nº 4, Page 172, line 36, it should read:

As it was expected, the time elapsed for the mixture to become unclottable by thrombin and extinction of paracoagulation differed accordingly with plasmin concentration; but the increase in NPN was the same for the 3 curves and reached $45.7 \mp 0.54 \times 10^{-3}$ mg N/ml of reaction mixture at the time of extinction. NPN was not determined at the time the mixture became unclottable by thrombin but it was calculated by interpolation to be equal for the three series and approximately 10×10^{-3} mg N/ml.

neighbouring tissues. Thus, a portion of artery, 10-12 cm long was obtained, completely excluded from the general circulation, but with complete nervous connections. This artery was perfused with or without glucose. Later, 80 I.U. of insulin were added; the effects on the general blood glucose level were observed. The following results were obtained:

1) Anesthesia with phenobarbital increases glycemia in 26 %.

2) Neither Tyrode alone nor with glucose, at a concentration of 2.1 mg %, when used as a perfusion liquid, is able to alter the blood glucose level.

3) If the excluded artery is perfused during 90 to 120 minutes with Tyrode without glucose which contains 80 I.U. of insulin, glycemia decreases about 12 %.

4) If the excluded artery is perfused with Tyrode plus glucose and 80 I.U. of insulin, the hypoglycemic effect is of about 26 %.

5) If the excluded artery is perfused during 30 minutes with a 2 % solution of Novocaine and then with Tyrode plus glucose and 80 I.U. of insulin, no hypoglycemic effect can be observed.

6) If vagotomy at the same side of the artery ha been performed, no hypoglycemic effect can be observed after perfusion of the excluded artery with Tyrode plus glucose and insulin.

7) In the pancreatectomized animal, the hypoglycemic effect of the perfusion with Tyrode and insulin is more intense.

The existence of angioceptors for insulin in the carotid artery is postulated, the sensitivity of which can be increased by augmenting the concentration of glucose in the perfusion fluid.

Alcohol metabolism in "drinking" and "non-drinking" rats. N. SEGOVIA DE RIQUELME, J. J. VITALE, D. M. HEGSTED AND J. MARDONES. *Instituto de Investigaciones sobre Alcoholismo and Instituto de Educación Física. Universidad de Chile. Department of Nutrition, Harvard School of Public Health, Boston, Mass.*

The rate of ethanol-1-C^{14} oxidation in rats with high voluntary alcohol consumption has been compared with those which consume voluntarily small amounts of alcohol. The difference observed in the amount of alcohol oxidized at three different doses (0.5; 2.0 and 3.0 g per Kilo of body weight) were non significant for the number of studied rats.